ARTICLE

OBC
www.rsc.org/obc

2-Hydroxymethyl-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (DRF-4367): an orally active COX-2 inhibitor identified through pharmacophoric modulation

Sunil Kumar Singh,*[a] Saibaba Vobbalareddy,[a] Srinivasa Rao Kalleda,[a] Shaikh Abdul Rajjak,[a] Seshagiri Rao Casturi,[b] Srinivasa Raju Datla,[b] Rao N. V. S. Mamidi,[b] Ramesh Mullangi,[b] Ravikanth Bhamidipati,[b] Rajagopalan Ramanujam,[b] Venkateswarlu Akella[b] and Koteswar Rao Yeleswarapu*[a]

[a] *Discovery Chemistry, Discovery Research-Dr. Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500 049, India. E-mail: sunilkumarsingh@drreddys.com; koteswarraoy@drreddys.com; Fax: +91-40-2304-5438/2304-5007; Tel: +91-40-2304-5439*

[b] *Discovery Biology, Discovery Research-Dr. Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500 049, India*

Received 24th February 2004, Accepted 22nd June 2004
First published as an Advance Article on the web 11th August 2004

Analogs of 1,5-diarylpyrazoles with a novel pharmacophore at N^1 were designed, synthesized and evaluated for the *in-vitro* cyclooxygenase (COX-1/COX-2) inhibitory activity. The variations at/around position-4 of the C-5 phenyl ring in conjunction with a CF_3 and CHF_2 groups at C-3 exhibited a high degree of potency and selectivity index (SI) for COX-2 inhibition. The *in-vivo* evaluation of these potent compounds with a few earlier ones indicated the 4-OMe-phenyl analog **6** and the 4-NHMe-phenyl analog **9** with a CF_3, and the 4-OEt-phenyl analog **19** with a CHF_2 group at C-3 to possess superior potency than celecoxib. In addition to its impressive anti-inflammatory, antipyretic, analgesic and anti-arthritic properties, compound **6** (DRF-4367) was found to possess an excellent pharmacokinetic profile, gastrointestinal (GI) safety in the long-term arthritis study and COX-2 potency in human whole blood assay. Thus, compound **6** was selected as an orally active anti-inflammatory candidate for pre-clinical evaluation.

Introduction

The two isoforms of prostaglandin (PG) synthase (cyclooxygenase, COX) exhibit tissue-dependent expression and regulation.[1] The COX-1, a constitutive enzyme, is primarily expressed in the gastrointestinal (GI) tract, and is responsible for the biosynthesis of PGs required for cytoprotection and platelet aggregation.[2] Hence, interference in its normal function for a long time leads to gastrointestinal toxicity such as ulceration, bleeding and perforation.[3] On the other hand, the isozyme COX-2, which is induced by the pro-inflammatory cytokines, *viz.* tumor necrosis factor-α (TNF-α), interleukines, mitogens and endotoxins in the inflammatory cells at the time of injury, plays a major role in the biosynthesis of PGs required by inflammatory cells (monocytes and macrophages) and causes inflammation, pain and fever.[4] The conventional non-steroidal anti-inflammatory drugs (NSAIDs), which are effective inhibitors of both COX-1 and COX-2, down regulate the biosynthesis of both kinds of PGs (cytoprotective and inflammatory) in most of the tissues and exhibit anti-inflammatory activity with side effects.[5] Thus, the selective inhibition of the isozyme COX-2, sparing COX-1, emerged as a novel approach in designing new anti-inflammatory agents with greater GI safety, and generated a new avenue in inflammation research. The clinical proof of this concept is evident from the launch of celecoxib[6] and rofecoxib[7] (Fig. 1) for the treatment of rheumatoid and osteo-arthritis. The more effective second generation drugs such as valdecoxib[8] and etoricoxib[9] further validated this new approach. The recent use of COX-2 inhibitors in other ailments like cancer[10] and Alzheimer's disease,[11] and further discovery of isozyme COX-3,[12] have put forth additional challenges and opportunities ahead. But, the recent reports have raised some safety issues over the use of COX-2 inhibitors towards a few vital organs.[13] However, this new way of treating inflammatory disorders has a great clinical advantage over the conventional NSAIDs, and still warrants a focused effort to have more efficacious COX-2 inhibitors with better patient acceptability.

In contrast to the diverse chemical scaffolds of traditional NSAIDs, COX-2 inhibitors are broadly represented by two chemical classes: (a) the diphenyl ethers, having the acidic methanesulfonamide ($MeSO_2NH$) group as the pharmacophore, exemplified by nimesulide;[14] and (b) the vicinal diaryl heterocycles having the 4-sulfamoyl (SO_2NH_2)-phenyl or methanesulfonyl (SO_2Me)-phenyl group as the pharmacophore, exemplified by celecoxib,[6] valdecoxib,[8] rofecoxib[7] and etoricoxib[9] (Fig. 1). The latter chemical class is well explored because of the COX-2 enzyme–ligand co-crystal structure available for the structure-based drug design.[15] The two vicinal phenyl rings of the COX-2 inhibitors orienting in a rigid *cis*-stilbene geometry along with the extension of the 4-SO_2NH_2/SO_2Me-phenyl ring towards the hydrophilic region of the COX-2 secondary pocket became the widely accepted feature for the selectivity. However, apart from vicinal diaryl carbocycles/heterocycles,[16] other scaffolds have also been reported recently.[17]

Nimesulide Celecoxib Valdecoxib Rofecoxib Etoricoxib

Fig. 1 COX-2 inhibitors.

With a view to achieving better COX-2 selectivity from known scaffolds,[18,19] we focused our attention on modulating the vital 4-sulfamoyl (SO_2NH_2)-phenyl ring of celecoxib.[20] During this exercise, it was observed that a hydroxymethyl group (CH_2OH) introduced adjacent to sulfonamide (SO_2NH_2) increased the COX-2 selectivity. Our idea of introducing the hydrophilic (CH_2OH) group

DOI: 10.1039/b402787f

This journal is © The Royal Society of Chemistry 2004

was based on the assumption that this would be preferred by the hydrophilic pocket of the COX-2 enzyme and lead to better inhibition. Herein, we report a brief SAR (structure–activity relationship) varying the substitution pattern at the C-3 and C-5 positions of 1,5-diarylpyrazole to optimize this new pharmacophore and obtain a better drug candidate with the minimum of effort. This report† also covers the comparative animal model study of these potent compounds with a few earlier ones[20] which finally led to the identification of a better drug candidate than celecoxib.

Results and discussion

Chemistry

The synthetic route to various 1,5-diarylpyrazoles **5–28** is depicted in Scheme 1. Simple coupling of the 3-hydroxymethyl-4-sulfamoylphenyl hydrazine hydrochloride **4** with the appropriate 1-phenyl-1,3-butanediones **3** in absolute ethanol with under heating afforded the desired analogs of 1,5-diarylpyrazole in very good yield (60–85%). Though the regiomeric bias was normally in favor of the 1,5-diarylpyrazoles, the minor, undesired regiomers were easily eliminated by triturating the product with a mixture of ethyl acetate–toluene after column chromatography. The formation of 1,5-diarylpyrazoles was identified by the appearance of a singlet for the C-4 proton at *ca.* 6.5 ppm in ^{1}H NMR. The required 1-phenyl-1,3-butanediones **3** were synthesized by Claisen condensation of the appropriate acetophenones **1** with ethyl trifluoroacetate and ethyl difluoroacetate **2**. This reaction was carried out under slightly modified condition using sodium hydride in dry DMF at a temperature of −5 to 30 °C to afford 1-phenyl-1,3-butanediones **3** in 95–98% yield. The commercially unavailable acetophenones were prepared according to the standard literature procedures.[6,21] The multi-step synthesis of the modified 3-hydroxymethyl-4-sulfamoylphenyl hydrazine hydrochloride **4** will be discussed elsewhere.[22] All compounds reported herein were characterized spectroscopically.

1 (Ar–CO–CH3) + 2 (R–CO–O–Et) —a, b→ [3: Ar–C(OH)=CH–CO–R ⇌ Ar–CO–CH=C(OH)–R]
3 + 4 (H_2NO_2S, CH_2OH, $NHNH_2.HCl$) —c→ minor + major
5 - 28

Scheme 1 *Reagents and conditions*: a) NaH, DMF, −5 to 30 °C, 4–5 h; b) dil. HCl; c) absolute ethanol, 50–60 °C, 5–6 h.

Biology

All of the new analogs of 1,5-diarylpyrazole synthesized here were initially screened at 10 μM for their selectivity against COX-1, obtained from the microsomal fraction of ram seminal vesicles, and against COX-2 recombinant human enzyme, expressed in Sf-9 cells infected with baculovirus. The promising compounds were further tested at lower concentrations. The enzyme activity was measured by a TMPD method and IC_{50}s were calculated using non-linear regression analysis of the percent inhibitions.[23] Celecoxib and indomethacin were used as reference standards for COX-2 selective and non-selective inhibitors respectively. Compounds with a higher selectivity index (SI), *i.e.* ratio of IC_{50}s (COX-1/COX-2), and a higher potency (lower IC_{50} for COX-2) were taken up for preliminary *in-vivo* anti-inflammatory screening at 30 mg kg^{-1} (po, *i.e.* oral administration) using the carrageenan-induced rat paw edema model.[24] ED_{50}s were calculated for the compounds exhibiting more than 50% inhibition in the rat paw volume. The potent compounds, selected based on these data, were further evaluated in different animal models[25–27] of inflammation-related disease after a single dose pharmacokinetic study at 10 mg kg^{-1} (po). The gastrointestinal safety profile of the most potent compound was assessed by the ^{51}Cr excretion test.[23b,28] Finally, the COX-2 selectivity of the potent compound was confirmed by human whole blood assay.[29]

The results of *in-vitro* COX-1/COX-2 enzyme inhibition exhibited by new analogs of 1,5-diarylpyrazole are depicted in Table 1 and 2. Few compounds such as **5**, **6** and **7** from our earlier report[20] have also been included here for the sake of comparison in the *in-vitro* as well as *in-vivo* studies. Since small hydrophobic groups around position-4 of the C-5 phenyl ring created a favorable environment for COX-2 potency and selectivity,[20] we planned to design few novel analogs comprising hitherto unreported groups in conjugation with a CF_3 group at C-3. We started this study by substituting small alkyl groups to the amino group of 4-aminophenyl analog **8**. Though 4-amino analog **8**, as expected, did not exhibit significant potency (6.600 μM), its 4-methylamino homologue **9**, was found to be highly potent (0.339 μM) and selective inhibitor of COX-2 enzyme. The 4-dimethylamino analog **10** was also found to be very potent (0.411 μM) but less selective whereas 4-ethylamino analog **11** remained as selective as **9** with lesser potency (0.744 μM). Since we observed a decreasing trend of potency in the amino analogs with the increase in size of the alkyl groups, a completely different amino derivative such as 4-azolanylphenyl analog **17** was synthesized to see if COX-2 enzyme has some pocket specificity around position-4 of the C-5 phenyl ring. Though this compound did not exhibit COX-2 potency like compounds **9–11**, it was still a selective inhibitor of COX-2 (37% at 1 μM) thereby confirming the presence of a specific hydrophobic area around position-4 of the phenyl ring for effective ligand-enzyme interaction. Few 3,4-disubstitutedphenyl derivatives such as the 3,4-dichloro analog **12** (0.405 μM), 3,4-dimethyl analog **13** (0.410 μM) and 3-methyl-4-methoxy analog **14** (0.560 μM) were also found to be highly potent. A cyclic prototype of compound **14**, 2,3-dihydrobenzo[b]furan-5-yl analog **15** turned out to be the most potent among the compounds reported here (0.228 μM). Similarly, its carbocyclic prototype 2,3-dihydro-1*H*-5-indenyl **16** which can also be visualized as the cyclic prototype of compound **13**, turned out to be highly potent (0.295 μM) and selective.

To compare the effect of CHF_2 and CF_3, we synthesized a few CHF_2 analogs of the potent compounds selected from Table 1 and few of our previously reported monosubstituted analogs.[20] The *in-vitro* results are shown in Table 2. Normally, the CHF_2 analogs were found to exhibit better potency than CF_3 analogs but this observation was limited only to smaller groups. The 4-chloro analog **20** (0.396 μM), 4-bromo analog **21** (0.458 μM) and 4-ethoxy analog **19** (0.708 μM) showed significantly improved potency over the parent CF_3 analogs.[20] But, a reverse trend was observed in the case of the 4-methylamino analog **23** and 4-dimethylamino analog **24**, which showed a drastic drop in COX-2 potency. Although the 3,4-dimethyl analog **25** (1.140 μM) showed a drop in potency when compared to its CF_3 analog **13**, the 3-methyl-4-methoxy analog **26** (0.793 μM; SI, 1261) exhibited the greatest selectivity among the two series (Tables 1 and 2). But, the 2,3-dihydro-1*H*-5-indenyl analog **28** (0.562 μM), a cyclic prototype of **25**, displayed an improved potency whereas the 2,3-dihydrobenzo[*b*]furan-5-yl analog **27**, a cyclic prototype of **26**, was found to be less COX-2 selective (SI, 55). However, the smaller hydrophobic groups, either a mono- or di-substituted phenyl, *e.g.* the 3-methoxy analog **18** (1.000 μM; SI, 450), and 3-chloro-4-fluoro analog **22** (0.653 μM; SI, 636) generally exhibited very good potency and selectivity.

Since the importance of the 4-sulfonamide-/methylsulfonylphenyl group in the vicinal diaryl heterocycles is well documented and the ligand–enzyme binding study has become a common tool to confirm the rational designs of COX-2 inhibitors,[16a] we wished to check whether these novel ligands also bind in the same fashion in the COX-2 pocket. The docking study of compound **6** has already been reported.[20] The binding modes of the most potent

† DRL Publication No. 283C.

Table 1 *In-vitro* data for 3-trifluoromethyl-1,5-diarylpyrazoles

Compound	Ar	IC_{50}/μM[a]		
		COX-1[b]	COX-2[c]	SI[d]
5	4-Me-phenyl	278.0	0.760	365
6	4-OMe-phenyl	63.0	0.365	172
7	4-SMe-phenyl	59.0	0.235	251
8	4-NH_2-phenyl	135.0	6.600	20
9	4-NHMe-phenyl	61.4	0.339	181
10	4-NMe_2-phenyl	50.0	0.411	121
11	4-NHEt-phenyl	136.5	0.744	183
12	3,4-Cl_2-phenyl	139.0	0.405	343
13	3,4-Me_2-phenyl	108.0	0.410	263
14	3-Me,4-OMe-phenyl	264.0	0.560	471
15		36.0	0.228	158
16		200.0	0.295	678
17	N	5[e] [f,g]	90[e] 37[f]	[g]
Celecoxib	—	10.75	0.076	141
Indomethacin	—	0.067	7.810	0.008

[a] Mean of three determinations. [b] COX-1 (obtained from ram seminal vesicles). [c] COX-2 (human, expressed in Sf-9-infected cells using baculovirus). [d] Selectivity Index (ratio of IC_{50}s, *i.e.* COX-1/COX-2). [e] % Inhibition at 10 μM concentration (one determination). [f] % Inhibition at 1 μM concentration (one determination). [g] Not determined.

Table 2 *In-vitro* data for 3-difluoromethyl-1,5-diarylpyrazoles

Compound	Ar	IC_{50}/μM[a]		
		COX-1[b]	COX-2[c]	SI[d]
18	3-OMe-phenyl	450.0	1.000	450
19	4-OEt-phenyl	>200.0[h]	0.708	>282[h]
20	4-Cl-phenyl	243.0	0.396	614
21	4-Br-phenyl	314.0	0.458	686
22	3-Cl,4-F-phenyl	415.0	0.653	636
23	4-NHMe-phenyl	12[e] [g]	91[e] 1.000	[g]
24	4-NMe_2-phenyl	100[e] [f,g]	100[e] 49[f]	[g]
25	3,4-Me_2-phenyl	134.0	1.140	118
26	3-Me,4-OMe-phenyl	>1000.0[h]	0.793	>1261[h]
27		36.7	0.663	55
28		186.0	0.562	331

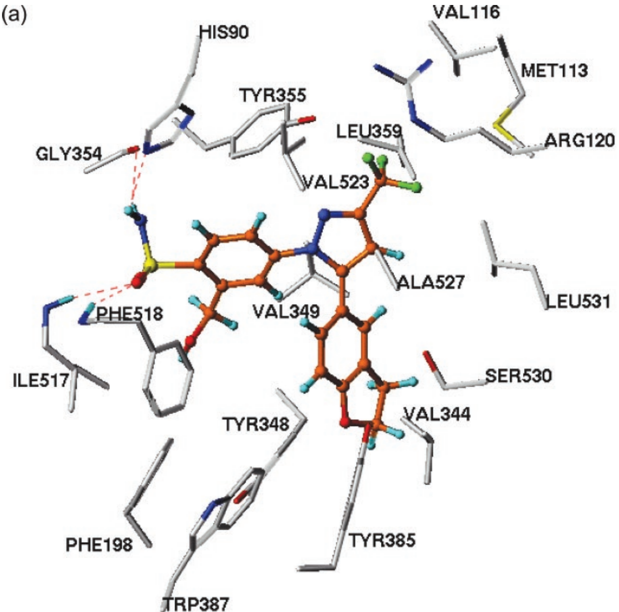
[a] Mean of three determinations. [b] COX-1 (obtained from ram seminal vesicles). [c] COX-2 (human, expressed in Sf-9-infected cells using baculovirus). [d] Selectivity Index (ratio of IC_{50}s, *i.e.* COX-1/COX-2). [e] % Inhibition at 10 μM concentration (one determination). [f] % Inhibition at 1 μM concentration (one determination). [g] Not determined. [h] > Indicates that precipitation was observed beyond this concentration, and IC_{50}s may be much higher than the reported values.

2,3-dihydrobenzo[*b*]furan-5-yl analog **15** (0.228 μM) and the least potent 4-amino analog **8** (6.600 μM) are shown in Fig. 2(a) and (b) respectively. In compound **15** [Fig. 2(a)], the two hydrogen atoms of the sulfonamide form hydrogen bonds with His-90 and Gly-354, whereas its two oxygen atoms form hydrogen bonds with the backbones of Phe-518 and Ile-517. The hydroxymethyl group introduced adjacent to the sulfonamide plays a crucial role in the ligand–enzyme interaction and binds in a region formed by Phe-518, Val-349 and Ile-517. The central pyrazole ring of all the ligands mentioned here is surrounded by Val-116, Val-349, Leu-359 and Ala-527. The CF_3 and CHF_2 groups normally bind in a pocket formed by Val-116, Val-349 and Arg-120. Fluorine atoms of these groups experience strong electrostatic interactions with the side chain of Arg-120. The C-5 phenyl ring binds in a large but narrow hydrophobic pocket of COX-2 formed by Tyr-348, Tyr-385, Trp-387 and Ser-530. The 4-amino group of compound **8** forms a hydrogen bond with Tyr-385 in the hydrophobic region, which is an unfavorable phenomenon and hence exhibits less potency [Fig. 2(b)]. The interaction and strain energies of a few docked ligands are shown in Table 3. Compound **8** has the highest strain energy and least interaction energy which supplements its lesser potency, whereas compound **15**, with the highest interaction energy, stands highly potent.

Based on the *in-vitro* potency criteria described, the potent compounds of Tables 1 and 2 and a few previously reported ones[20] were screened in the carrageenan-induced rat paw edema model at 30 mg kg^{-1} (po). Many compounds reduced the edema and exhibited anti-inflammatory activity in this preliminary animal model. Compounds exhibiting *ca.* 50% inhibition of paw edema were further tested at 1, 3, 10 and 30 mg kg^{-1} in six animals per group to calculate the ED_{50}s. These results are depicted in Table 4. Though many compounds displayed very good potency at 30 mg kg^{-1}, very few were found to be better than celecoxib based on the ED_{50} values. The *in-vitro* potent compounds like 4-methylsulfanyl analog **7**, 3,4-dichloro analog **12**, 3-methyl-4-methoxy analogs **14** and **26**, 2,3-dihydro-1*H*-5-indenyl analog **16**, 3-methoxy analog **18**, 4-bromo analog **21**, 3-chloro-4-fluoro analog **22** and 2,3-dihydrobenzo[b]furan-5-yl analog **15** failed to show the expected efficacy in animal model. Though the exact reason was not explored, their poor pharmacokinetic properties and metabolic instability were speculated as the main cause of this failure. The compounds having CHF_2 group at position-3 of the pyrazole ring in conjugation with small groups at position-4 of C-5 phenyl ring, even being highly potent *in-vitro*, did not show satisfactory *in-vivo* efficacy except the 4-ethoxy analog **19** which showed ED_{50} of 4.5 mg kg^{-1}. This could possibly be due to the decrease in number of the most electronegative fluorine atom which might have caused a serious deviation from the normal arrangement in the COX-2 pocket at physiological condition. The compounds such as 4-methoxy analog **6** (1.9 mg kg^{-1}), 4-methylamino analog **9** (3.5 mg kg^{-1}) and 4-ethoxy analog **19** (4.5 mg kg^{-1}) having better efficacy than celecoxib in the preliminary screening,[24] were selected for other inflammation-related animal studies.

The single dose comparative pharmacokinetic data for the active compounds **6**, **9** and **19**, taking celecoxib as the standard at 10 mg kg^{-1} (po), is mentioned in Table 5, and the graph is shown in Fig. 3. While compounds **9** and **19** exhibited almost similar pharmacokinetic profiles as celecoxib, **6** showed the maximum drug concentration in blood (AUC_{0-t}, 19.40 μg.h.mL^{-1}) with a better elimination half life ($t_{1/2}$, 7.01 h) when compared to celecoxib (AUC_{0-t}, 9.68 μg.h.mL^{-1}; $t_{1/2}$, 6.87 h). However, the time to reach the maximum concentration in blood, a criteria for the onset of activity (T_{max}, 3 h), was found to be the same for these compounds like that for celecoxib. Though the overall pharmacokinetic profile of compound **6** was found to be the best among all, other two compounds **9** and **19** were also studied in the next animal models of inflammation.

The comparative data obtained from different animal models is depicted in Table 6. The above three compounds were initially screened at 30 mg kg^{-1} (po) in the endotoxin-induced pyresis model for assessing their antipyretic activity.[25] Though all three



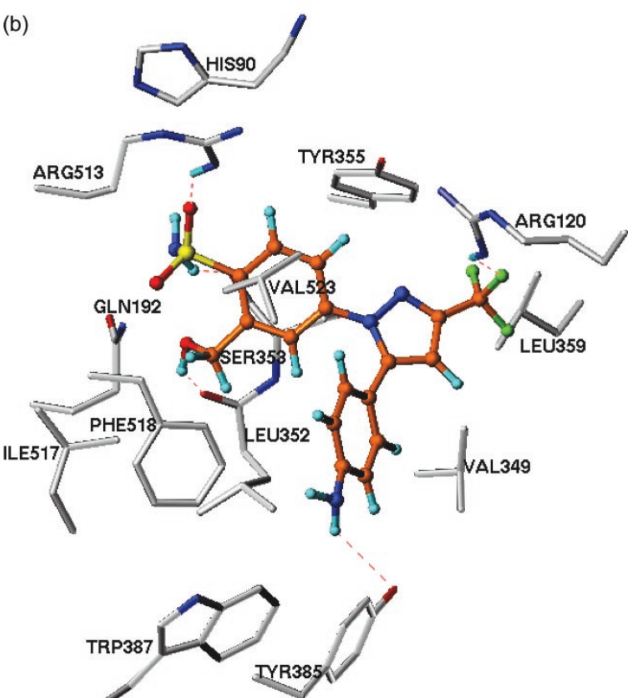


Fig. 2 Docking of (a) the most active compound **15** and (b) the least active compound **8** in the active site of murine COX-2 enzyme. Ligand structures are shown in ball and stick model where carbon atoms are shown in orange. Hydrogen and backbone atoms are not shown for clarity.

Table 3 Interaction and strain energies of 1,5-diarylpyrazoles

Compound	Interaction energy/kcal mol^{-1}	Strain energy/kcal mol^{-1}
8	−35.228	4.898
11	−38.919	1.044
15	−39.257	1.899
19	−39.188	1.117
20	−38.22	1.789

compounds were active at this dose, compounds **6** and **9** were found to have ED_{50}s of 4.68 and 3.53 mg kg^{-1} respectively, were found to be four times better than celecoxib (15.68 mg kg^{-1}). We again reasoned that the CHF_2 group was behind the failure of compound **19** in the disease condition. Similarly, when these three compounds were screened in the carrageenan-induced hyperalgesia model to assess their analgesic activity,[26] compound **6** (1.13 mg kg^{-1}) was

Table 4 *In-vivo* data for 1,5-diarylpyrazoles in the carrageenan-induced rat paw edema model

Compound	% Reduction in paw vol. (30 mg kg^{-1})[a]	ED_{50}/mg kg^{-1} [b]
5	63	10.0
6	60	1.9
7	47	[c]
8	56	25.2
9	63	3.5
12	50	>30
14	52	21.7
15	55	22.3
16	54	23.5
18	61	25.4
19	70	4.5
20	61	7.9
21	42	[c]
22	50	>30
26	60	18.5
Celecoxib	53	6.7

[a] Results of single experiment carried out taking six animals per group (male wistar rats) on oral dosing. [b] The experiment was carried out in eight animals per group (male wistar rats) on oral dosing of 1, 3, 10 and 30 mg kg^{-1}, and ED_{50}s are the average of three experiments. [c] No dose response.

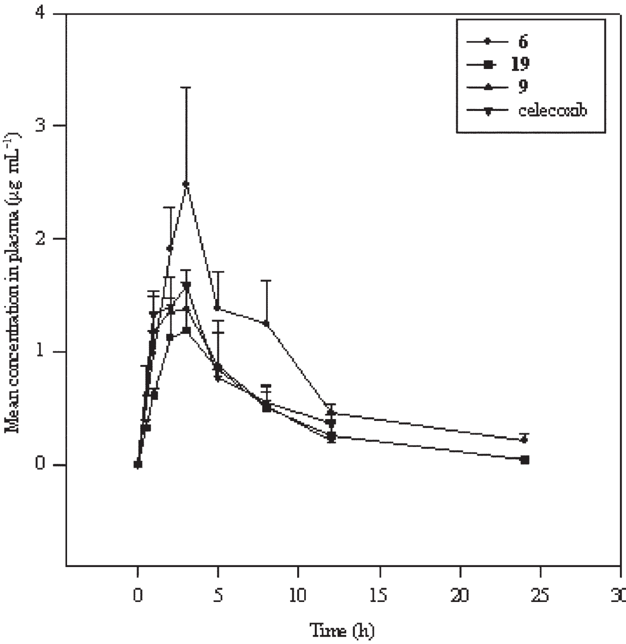


Fig. 3 Oral pharmacokinetics of 1,5-diarylpyrazoles in male wistar rats at 10 mg kg^{-1} (single dose).

found to be better than celecoxib (2.11 mg kg^{-1}). While compound **19** was found to be equipotent to celecoxib, compound **9** was relatively less potent. Since a reasonable balance of antipyretic and analgesic activity is required with the anti-inflammatory properties in this therapeutic area, it was decided at this stage to study only compounds **6** and **9** in the arthritis model. In a 21 day adjuvant-induced prophylactic model of arthritis,[27] compound **6** (0.11 mg kg^{-1}) was found to be almost seven fold more potent than celecoxib (0.70 mg kg^{-1}). Compound **9** (0.62 mg kg^{-1}) was found to have the same potency as celecoxib in this study. Even in a 30 day adjuvant-induced curative model of arthritis (treatment commenced on the fifteenth day after establishing the disease by adjuvant injection), compound **6** (<0.30 mg kg^{-1}) was found to be almost equipotent to celecoxib (0.13 mg kg^{-1}). Compound **9** in this study was found to be less potent (5.20 mg kg^{-1}) than compound **6** and celecoxib.

Finally, the preliminary gastrointestinal safety of compound **6** was assessed by ^{51}Cr excretion tests both in acute as well as in chronic models of arthritis using indomethacin and celecoxib as control drugs.[23b,28] This compound was found to be as safe as celecoxib. The data are presented in Table 7. Compound **6** was also tested for COX-2 selectivity in human whole blood where it was

Table 5 Oral pharmacokinetic profiles of selected 1,5-diarylpyrazoles in wistar rats at 10 mg kg^{-1} [a]

Parameters	6	9	19	Celecoxib
$AUC_{(0-t)}$ [b] (μg.h.mL^{-1})	19.40 ± 2.99	9.06 ± 1.44	9.75 ± 3.02	9.68 ± 0.63
$AUC_{(0-\infty)}$ [b] (μg.h.mL^{-1})	21.61 ± 1.96	10.48 ± 2.82	10.03 ± 2.94	13.53 ± 2.88
C_{max} [c] (μg mL^{-1})	2.55 ± 0.83	1.42 ± 0.21	1.21 ± 0.39	1.63 ± 0.16
T_{max} [d] (h)	2.75 ± 0.50	3.25 ± 1.26	2.75 ± 0.50	2.80 ± 0.45
K_{el} [e] (h^{-1})	0.11 ± 0.03	0.26 ± 0.13	0.15 ± 0.04	0.11 ± 0.03
$t_{1/2}$ [f] (h)	7.01 ± 1.93	3.40 ± 2.07	4.81 ± 1.14	6.87 ± 2.65

[a] Average of two experiments, each carried out in the group of five animals on single dosing. [b] Area under curve. [c] Peak plasma concentration. [d] Time taken in achieving C_{max}. [e] Terminal elimination constant. [f] Terminal half-life.

Table 6 *In-vivo* data for selected 1,5-diarylpyrazoles in different animal models

	ED$_{50}$/mg kg^{-1}				
				Rat adjuvant arthritis	
Compound	Paw edema [a]	Pyresis [b]	Hyperalgesia [b]	Prophylactic [c]	Curative [d]
6	1.80 ± 0.08	4.68 ± 0.82	1.13 ± 0.06	0.11	<0.30
9	3.12 ± 0.28	3.53	7.44 ± 1.27	0.62	5.20
19	5.62 ± 1.11	>30	2.02	[e]	[e]
Celecoxib	6.65 ± 0.98	15.68	2.11 ± 0.57	0.70	0.13

[a] The experiment was carried out in six animals per group (male wistar rats) on oral dosing of 1, 3, 10 and 30 mg kg^{-1}, and ED$_{50}$s are the average of three experiments. [b] ED$_{50}$s are the average of two experiments carried out with six animals per group on oral dosing of 1, 3, 10 and 30 mg kg^{-1}. [c] Value from 21 days experiment with eight animals per group (see the experimental section). [d] Value from 30 days experiment carried with eight animals per group (see the experimental section). [e] Not determined.

Table 7 ^{51}Cr excretion test for compound **6** in wistar rats

		% ^{51}Cr excretion	
Compound	Dose/mg kg^{-1}	Acute dosing [a]	Chronic dosing [b]
Control	—	0.41 ± 0.01	0.70 ± 0.09
6	100	0.43 ± 0.04	0.64 ± 0.1
Celecoxib	100	0.31 ± 0.05	0.63 ± 0.07
Indomethacin	100	1.95 ± 0.36	Died

[a] Experiments were performed on a group of eight animals for 48 h. The values are the average of two experiments. [b] Experiments were performed on a group of eight animals for 14 days. The values are the average of two experiments.

Table 8 Human whole blood assay of compound **6**

	IC$_{50}$/μM [a]		
Compound	COX-1	COX-2	Ratio (COX-1/COX-2)
6 (DRF-4367)	5.38 ± 1.1	0.04 ± 0.007	125
Celecoxib	8.4 ± 1.6	0.14 ± 0.005	62

[a] All values are given as the mean ± SE (n = 6).

found to be more potent (0.04 μM) and selective (SI, 125) than the standard drug celecoxib (0.14 μM; SI, 62; Table 8).[29] Thus, based on the results described above, compound **6** was selected as a potent and safe candidate for further pre-clinical studies.

Conclusion

In this report, we described the design and synthesis of a few 1,5-diarylpyrazoles having a novel pharmacophore at N^1. The *in-vitro* as well as *in-vivo* evaluation of their C-3 and C-5 substituted analogs afforded several potent compounds, *viz.* 4-OMe-phenyl and 4-NHMe-phenyl analogs **6** and **9**, both with CF_3, and 4-OEt-phenyl analog **19** with a CHF_2 group at the 3-position. Of these, **6** (DRF-4367) was found to be superior to celecoxib based on the anti-inflammatory, antipyretic, analgesic and anti-arthritic potential. Apart from its excellent potency, it has exhibited a very high concentration of the drug in systemic circulation upon oral dosing. This compound displayed GI safety like celecoxib during the long-term arthritis study and a far superior COX-2 selectivity in human whole blood assay. Based on the above data, compound **6** is selected as the orally active candidate for further pre-clinical evaluation.

Experimental

Chemistry protocols

Solvents, except LR grade, were distilled before use. Research chemicals such as acetophenones and halo-esters were either purchased from Lancaster/Aldrich Co. and used without further purification in the reactions, or were prepared according to the procedure described in the literature.[6,21] Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck), visualizing with ultraviolet light or iodine spray. Usually, the flash column chromatographic purification was performed over 100–200 or 230–400 mesh silica gel using a mixture of ethyl acetate and petroleum ether. While the standard sample of celecoxib was prepared according to the literature procedure,[6] the standard drug indomethacin was isolated from capsules bought from the medical stores. The yields of the products reported here are un-optimized. Melting points were determined on Buchi melting point B-540 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1650 spectrometer. ^{1}H and ^{13}C NMR experiments were respectively performed at 200 MHz and 50 MHz on a Varian Gemini 200 spectrometer and their chemical shifts are reported in δ units with respect to TMS as the internal standard. Mass spectra were recorded on a HP-5989A spectrometer. Elemental analysis was carried out for C, H, N using a Perkin-Elmer 2400 series II CHN–O analyzer. All of the analyses were performed by the Analytical Research group of Discovery-Research, Dr. Reddy's Laboratories Ltd. The purity of the final compounds were determined by HPLC using 'System 1' which consisted of a Hichrom RPB (250 mm) column, and mobile phase 0.01 M KH_2PO_4/CH_3CN (50 : 50), and 'System 2' which comprised an Intersil ODS 3 V (250 mm) column, and mobile phase H_2O/CH_3CN (50 : 50). Both systems were run at 1.0 mL min^{-1} with UV detection at the wavelength of maximum absorption.

General procedure for the preparation of 1,5-diarylpyrazoles

Step 1. General procedure for the preparation of acetophenone 1. Representative preparation of 1-(2,3-dihydro-1*H*-5-indenyl)-1-ethanone. Acetyl chloride (3.77 mL, 52.86 mmol) was introduced to a suspension of anhydrous aluminium chloride

(7.33 g, 55.00 mmol) in dichloromethane (75 mL) under an argon atmosphere at 0–5 °C. After stirring the reaction mixture for 0.5 h at this temperature, indan (5.2 mL, 42.30 mmol) was slowly added over a period of 15 min. After maintaining the reaction mixture at this temperature for 2 h, it was allowed to stir at room temperature for the next 3 h and poured over crushed ice. It was extracted with dichloromethane (3 × 50 mL), and the combined organic layers after washing with water were dried (anhydrous Na_2SO_4) and evaporated to yield an oil which was purified by column chromatography using 1% ethyl acetate–petroleum ether to afford the viscous liquid of the title compound (5.42 g, 80%). This compound was used in the next step without further purification. Bp 85 °C (0.5 mm) [lit.[21] 80 °C (0.2 mm)]. IR (neat) 2954, 1680, 1608, 1424, 1269 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.82 (s, 1H, Ar), 7.76 (d, J = 7.8 Hz, 1H, Ar), 7.29 (d, J = 8.0 Hz, 1H, Ar), 2.95 (t, J = 7.4 Hz, 4H, 2 × CH_2), 2.59 (s, 3H, $COCH_3$), 2.21–2.05 (m, 2H, CH_2). MS (DIP) 160 (M^+), 145 (M − 15, 100%)$^+$, 117, 115, 91.

Step 2. General procedure for the preparation of 1-phenyl-1,3-butanediones 3. Representative preparation of 1-(2,3-dihydro-1*H*-5-indenyl)-4,4,4-trifluoro-1,3-butanedione. 1-(2,3-Dihydro-1*H*-5-indenyl)-1-ethanone (5.0 g, 31.25 mmol), prepared in step 1, was dissolved in 25 mL of dry DMF under an argon atmosphere and 60% NaH (1.56 g, 39.06 mmol) was added in three lots whilst maintaining the temperature between −5 and 0 °C. After stirring at this temperature for 0.5 h, ethyl trifluoroacetate (4.64 mL, 39.06 mmol) was injected into the reaction mixture and was allowed to stir at ambient temperature for 4–5 h. The reaction mixture was poured into ice water, acidified with 2 M HCl and extracted with ethyl acetate. The combined organic layers were washed with water, dried (anhydrous Na_2SO_4) and evaporated to obtain a residue which was finally purified by column chromatography using 2% ethyl acetate–petroleum ether to yield a light yellow solid of the title compound (5.2 g, 65%) which was used as such for the next step. Mp 41–42 °C. IR (KBr) 3475, 2958, 1570, 1459 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.81 (s, 1H, Ar), 7.75 (d, J = 8.0 Hz, 1H, Ar), 7.34 (d, J = 7.6 Hz, 1H, Ar), 6.55 (s, 1H, CH), 2.98 (t, J = 7.2 Hz, 4H, 2 × CH_2), 2.22–2.10 (m, 2H, CH_2). MS (DIP) 256 (M^+), 228, 187 (100%), 145, 115, 91.

Step 3. Representative preparation of 4-[5-(2,3-dihydro-1*H*-5-indenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-2-hydroxymethyl-1-benzenesulfonamide 16. 3-Hydroxymethyl-4-sulfamoylphenyl hydrazine hydrochloride **4** (5.08 g, 23.43 mmol) was dissolved in MeOH (30 mL) under an argon atmosphere and acidified to pH 1–2 using IPA–HCl. The reaction mixture was stirred at room temperature for 0.5 h and the solvent was completely removed under high vacuum at 40–50 °C. The solid obtained was dissolved in absolute alcohol (50 mL) and an ethanolic solution of the above-prepared 1-(2,3-dihydro-1*H*-5-indenyl)-4,4,4-trifluoro-1,3-butanedione (5.0 g, 19.53 mmol) was added at room temperature. After heating at 50–60 °C for 10–12 h under an argon atmosphere, the reaction mixture was concentrated, stirred with ice-cold water and extracted with ethyl acetate. The combined organic layers were washed with water, dried and evaporated. The residue obtained was purified by column chromatography using 25% ethyl acetate–petroleum ether to yield a gummy material which upon trituration with ethyl acetate–toluene afforded the title compound **16** as a colorless solid (5.55 g, 65%). Mp 118–120 °C. IR (KBr) 3323, 1602 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.00 (d, J = 8.4 Hz, 1H, Ar), 7.69 (s, 1H, Ar), 7.35–7.25 (m, 2H, Ar), 7.14 (s, 1H, Ar), 6.96 (d, J = 8.0 Hz, 1H, Ar), 6.74 (s, 1H, C_4H), 5.40 (bs, 2H, SO_2NH_2), 5.00 (s, 2H, CH_2O), 3.00–2.80 (m, 4H, 2 × CH_2), 2.60 (bs, 1H, OH), 2.20–2.00 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 145.7, 145.4, 144.6, 142.7, 142.2 (q, J = 37.3 Hz, 1C, C-4), 141.5, 139.8, 128.0, 126.9, 126.1, 125.8 (q, J = 266.5 Hz, 1C, CF_3), 124.7, 124.6, 123.8, 123.2, 106.1, 59.4, 32.3 (2C), 24.9. MS (CI method) 437 (M^+), 419, 338. HPLC (System 1) 96.63%; (System 2) 97.43%. Anal. ($C_{20}H_{18}F_3N_3O_3S$) C: calc., 54.92; found, 55.25; H: calc., 4.15; found, 3.91; N: calc., 9.61; found, 9.75.

Compound 6. This was prepared from 4-methoxyacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 85%. Mp 188–189 °C. IR (neat) 3311, 1609 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.97 (d, J = 8.6 Hz, 1H, Ar), 7.62 (d, J = 1.8 Hz, 1H, Ar), 7.25 (d, J = 8.0 Hz, 1H, Ar), 7.15 (d, J = 8.4 Hz, 2H, Ar), 6.88 (d, J = 8.6 Hz, 2H, Ar), 6.71 (s, 1H, C_4H), 5.43 (bs, 2H, SO_2NH_2), 5.01 (d, J = 3.8 Hz, 2H, CH_2O), 3.83 (s, 3H, OCH_3), 2.65 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 160.1, 145.1, 142.8, 142.0 (q, J = 35.5 Hz, 1C, C-4), 141.5, 139.9, 130.3 (2C), 128.1, 123.9, 123.3, 121.5 (q, J = 267.4 Hz, 1C, CF_3), 120.6, 114.4 (2C), 105.9, 59.3, 55.3. MS (CI method) 428 (M + 1)$^+$, 409, 392, 346. HPLC (System 1) 98.35%. Anal. ($C_{18}H_{16}F_3N_3O_4S$) C: calc., 50.58; found, 50.27; H: calc., 3.77; found, 3.92; N: calc., 9.83; found, 9.49.

Compound 8. The compound was obtained by the acidic hydrolysis of the corresponding acetamide (not reported here), prepared from N^1-(4-acetylphenyl) acetamide and ethyl trifluoroacetate following steps 2 and 3. Yield 65%. Mp 210–212 °C. IR (KBr) 3383, 3251, 1615, 1478 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.85 (d, J = 8.2 Hz, 1H, Ar), 7.75 (s, 1H, Ar), 7.60 (bs, 1H, OH), 7.25 (d, J = 8.2 Hz, 1H, Ar), 6.99 (s, 1H, C_4H), 6.93 (d, J = 8.2 Hz, 2H, Ar), 6.51 (d, J = 8.2 Hz, 2H, Ar), 5.49 (bs, 2H, SO_2NH_2), 4.90 (s, 2H, CH_2O). MS (CI method) 412 (M^+), 395, 331, 303. HPLC (System 1) 96.65%; (System 2) 97.51%. Anal. ($C_{17}H_{15}F_3N_4O_3S$) C: calc., 49.51; found, 49.79; H: calc., 3.67; found, 3.88; N: calc., 13.59; found, 13.22.

Compound 9. This compound was prepared from 4-methylaminoacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 78%. Mp 188–190 °C. IR (KBr) 3414, 3301, 1615 cm^{-1}. ^1H NMR (CD_3OD): δ 7.95 (d, J = 8.8 Hz, 1H, Ar), 7.83 (s, 1H, Ar), 7.26 (dd, J = 8.4, 1.8 Hz, 1H, Ar), 7.00 (d, J = 8.8 Hz, 2H, Ar), 6.76 (s, 1H, C_4H), 6.53 (d, J = 8.8 Hz, 2H, Ar), 5.00 (s, 2H, CH_2O), 2.74 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 150.6, 146.3, 142.80, 142.4 (q, J = 35.6 Hz, 1C, C-4), 142.1, 139.8, 129.8 (2C), 128.1, 124.0, 123.4, 121.5 (q, J = 267.1 Hz, 1C, CF_3), 114.8, 111.6 (2C), 104.7, 59.6, 29.4. MS (CI method) 426 (M^+), 408, 345. HPLC (System 1) 99.30%. Anal. ($C_{18}H_{17}F_3N_4O_3S$) C: calc., 50.70; found, 51.05; H: calc., 4.02; found, 3.82; N: calc., 13.14; found, 12.89.

Compound 10. Preparation was from 4-dimethylaminoacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 75%. Mp 178–180 °C. IR (KBr) 3418, 3318, 1611, 1477 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.96 (d, J = 8.6 Hz, 1H, Ar), 7.65 (d, J = 2.0 Hz, 1H, Ar), 7.27–7.23 (m, 1H, Ar), 7.05 (d, J = 8.8 Hz, 2H, Ar), 6.65 (d, J = 8.6 Hz, 2H, Ar), 6.61 (s, 1H, C_4H), 5.43 (bs, 2H, SO_2NH_2), 5.03 (s, 2H, CH_2O), 2.99 (s, 6H, 2 × CH_3), 2.65 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 150.5, 146.0, 142.8, 142.6 (q, J = 36.4 Hz, 1C, C-4), 141.9, 139.8, 129.5 (2C), 128.8, 123.9, 123.3, 120.5 (q, J = 267.4 Hz, 1C, CF_3), 115.0, 111.8 (2C), 104.9, 59.4, 39.7 (2C). MS (CI method) 440 (M^+), 421, 358. HPLC (System 1) 96.77%; (System 2) 97.41%. Anal. ($C_{19}H_{19}F_3N_4O_3S$) C: calc., 51.81; found, 51.93; H: calc., 4.35; found, 4.66; N: calc., 12.72; found, 12.75.

Compound 11. Preparation was from 4-ethylaminoacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 72%. Mp 171–172 °C. IR (KBr) 3406, 1616, 1451 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.00 (d, J = 8.2 Hz, 1H, Ar), 7.67 (s, 1H, Ar), 7.30 (d, J = 8.8 Hz, 1H, Ar), 7.02 (d, J = 8.8 Hz, 2H, Ar), 6.68 (s, 1H, C_4H), 6.56 (d, J = 8.8 Hz, 2H, Ar), 5.54 (bs, 2H, SO_2NH_2), 5.05 (d, J = 3.8 Hz, 2H, CH_2O), 3.18 (q, J = 7.4 Hz, 2H, CH_2CH_3), 2.65 (bs, 1H, OH), 1.30 (t, J = 6.8 Hz, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 149.6, 146.2, 142.6, 142.2 (q, J = 36.2 Hz, 1C, C-4), 141.9, 139.6, 129.6 (2C), 127.9, 123.8, 123.2, 121.2 (q, J = 267.1 Hz, 1C, CF_3), 114.6, 111.7 (2C), 104.6, 59.4, 37.1, 14.3. MS (CI method) 441 (M + 1)$^+$, 423. HPLC (System 2) 97.80%. Anal. ($C_{19}H_{19}F_3N_4O_3S$) C: calc., 51.81; found, 51.79; H: calc., 4.35; found, 4.48; N: calc., 12.72; found, 12.87.

Compound 12. Preparation was from 3,4-dichloroacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 60%. Mp 148–150 °C. IR (KBr) 3454, 3241, 1603 cm^{-1}. ^1H NMR ($CDCl_3$):

δ 7.99 (d, $J = 8.4$ Hz, 1H, Ar), 7.63 (d, $J = 2.0$ Hz, 1H, Ar), 7.44–7.39 (m, 2H, Ar), 7.22 (dd, $J = 8.6, 2.2$ Hz, 1H, Ar), 6.93 (dd, $J = 8.0, 2.0$ Hz, 1H, Ar), 6.78 (s, 1H, C_4H). 5.43 (bs, 2H, SO_2NH_2), 5.0 (d, $J = 3.8$ Hz, 2H, CH_2O), 2.74 (bs, 1H, OH). MS (CI method) 466 (M^+), 447, 430, 384. HPLC (System 1) 96.43%; (System 2) 97.85%. Anal. ($C_{17}H_{12}Cl_2F_3N_3O_3S$) C: calc., 43.79; found, 43.55; H: calc., 2.59; found, 2.65; N: calc., 9.01; found, 9.25.

Compound 13. Preparation was from 3,4-dimethylacetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 58%. Mp 132–134 °C. IR (KBr) 3522, 1605, 1472 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.95 (d, $J = 8.4$ Hz, 1H, Ar), 7.66 (s, 1H, Ar), 7.25 (d, $J = 7.8$ Hz, 1H, Ar), 7.16 (m, 2H, Ar), 6.86 (d, $J = 7.4$ Hz, 1H, Ar), 6.74 (s, 1H, C_4H), 5.50 (bs, 2H, SO_2NH_2), 5.03 (s, 2H, CH_2O), 2.80 (bs, 1H, OH), 2.28 (s, 3H, CH_3), 2.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 145.4, 142.8, 142.2 (q, $J = 37.5$ Hz, 1C, C-4), 141.5, 140.0, 137.9, 137.1, 129.8 (2C), 128.0, 126.2, 125.8, 123.9, 123.3, 121.5 (q, $J = 266.8$ Hz, 1C, CF_3), 106.1, 59.4, 19.3, 19.2. MS (CI method) 425 (M^+), 407, 379, 327. HPLC (System 1) 97.55%; (System 2) 97.45%. Anal. ($C_{19}H_{18}F_3N_3O_3S$) C: calc., 53.64; found, 53.48; H: calc., 4.26; found, 4.68; N: calc., 9.88; found, 10.21.

Compound 14. Preparation was from 3-methyl-4-methoxy acetophenone and ethyl trifluoroacetate following steps 2 and 3. Yield 80%. Mp 156–158 °C. IR (KBr) 3422, 3317, 1610, 1472 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.98 (d, $J = 8.6$ Hz, 1H, Ar), 7.65 (d, $J = 2.2$ Hz, 1H, Ar), 7.24 (d, $J = 8.4$ Hz, 1H, Ar), 7.07 (s, 1H, Ar), 6.90 (d, $J = 2.0$ Hz, 1H, Ar), 6.76 (d, $J = 8.4$ Hz, 1H, Ar), 6.70 (s, 1H, C_4H), 5.42 (bs, 2H, SO_2NH_2), 5.00 (s, 2H, CH_2O), 3.85 (s, 3H, OCH_3), 2.65 (bs, 1H, OH), 2.19 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 158.1, 145.3, 142.6, 142.1 (q, $J = 37.6$ Hz, 1C, C-4), 141.5, 139.8, 130.9, 127.9, 127.8, 126.3, 123.8, 123.2, 122.2 (q, $J = 268.2$ Hz, 1C, CF_3), 120.1, 110.4, 105.7, 59.2, 55.4, 15.9. MS (CI method) 441 (M^+), 422, 343. HPLC (System 1) 99.00%; (System 2) 98.89%. Anal. ($C_{19}H_{18}F_3N_3O_4S$) C: calc., 51.70; found, 50.98; H: calc., 4.11; found, 3.88; N: calc., 9.52; found, 9.01.

Compound 15. Preparation was from 2,3-dihydrobenzo[*b*]furan and ethyl trifluoroacetate following steps 1–3. Yield 77%. Mp 140–142 °C. IR (KBr) 3337, 1614, 1469 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.02 (d, $J = 8.2$ Hz, 1H, Ar), 7.68 (d, $J = 1.8$ Hz, 1H, Ar), 7.26 (d, $J = 7.0$ Hz, 1H, Ar), 7.10 (s, 1H, Ar), 6.97 (d, $J = 6.6$ Hz, 1H, Ar), 6.77 (d, $J = 8.4$ Hz, 1H, Ar), 6.71 (s, 1H, C_4H), 5.46 (bs, 2H, SO_2NH_2), 5.05 (s, 2H, CH_2O), 4.65 (t, $J = 8.8$ Hz, 2H, OCH_2CH_2), 3.22 (t, $J = 8.4$ Hz, 2H, OCH_2CH_2), 2.77 (bs, 1H, OH). MS (CI method) 439 (M^+), 421, 356. HPLC (System 1) 98.93%. Anal. ($C_{19}H_{16}F_3N_3O_4S$) C: calc., 51.94; found, 51.83; H: calc., 3.67; found, 3.79; N: calc., 9.56; found, 9.80.

Compound 17. Preparation was from 1-phenylazolane and ethyl trifluoroacetate following steps 1–3. Yield 67%. Mp 186–188 °C. IR (KBr) 3413, 1611, 1450 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.96 (d, $J = 8.8$ Hz, 1H, Ar), 7.67 (s, 1H, Ar), 7.26 (s, 1H, Ar), 7.02 (d, $J = 8.2$ Hz, 2H, Ar), 6.65 (s, 1H, C_4H), 6.48 (d, $J = 8.2$ Hz, 2H, Ar), 5.45 (bs, 2H, SO_2NH_2), 5.03 (s, 2H, CH_2O), 3.29–3.20 (m, 4H, 2 × NCH_2CH_2), 2.05–2.00 (m, 4H, 2 × NCH_2CH_2). MS (CI method) 466 (M^+, 100%), 448, 384, 356. HPLC (System 1) 99.75%. Anal. ($C_{21}H_{21}F_3N_4O_3S$) C: calc., 54.07; found, 53.88; H: calc., 4.54; found, 4.91; N: calc., 12.01; found, 12.04.

Compound 18. Preparation was from 3-methoxyacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 68%. IR (KBr) 3249, 1603, 1481, 1427 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.94 (d, $J = 8.3$ Hz, 1H, Ar), 7.61 (s, 1H, Ar), 7.33–7.18 (m, 2H, Ar), 7.08–6.77 (m, 3H, Ar), 6.77 (t, $J = 54.6$ Hz, 1H, CHF_2), 6.62 (s, 1H, C_4H), 5.45 (bs, 2H, SO_2NH_2), 5.00 (d, $J = 3.8$ Hz, 2H, CH_2O), 3.78 (s, 3H, OCH_3), 2.75 (bs, 1H, OH). MS (CI method) 409 (M^+), 389, 356, 328. HPLC (System 1) 99.31%. Anal. ($C_{18}H_{17}F_2N_3O_4S$) C: calc., 52.81; found, 52.93; H: calc., 4.19; found, 4.50; N: calc., 10.26; found, 10.53.

Compound 19. Preparation was from 4-ethoxyacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 76%. Mp 150–152 °C. IR (KBr) 3453, 3320, 3214, 1607, 1461 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.97 (d, $J = 8.5$ Hz, 1H, Ar), 7.58 (d, $J = 2.0$ Hz, 1H, Ar), 7.24–7.10 (m, 4H, Ar), 6.89–6.68 (m, 2H, Ar), 6.66 (t, $J = 54.0$ Hz, 1H, CHF_2), 5.45 (bs, 2H, SO_2NH_2), 5.00 (s, 2H, CH_2O), 4.03 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 2.78 (bs, 1H, OH), 1.42 (t, $J = 7.0$ Hz, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 159.1, 147.2 (t, $J = 28.8$ Hz, 1C, C-4), 144.6, 142.6, 141.8, 139.3, 130.2 (2C), 128.0, 123.6, 122.8, 121.0, 114.7 (2C), 111.4 (t, $J = 231.4$ Hz, 1C, CHF_2), 105.2, 63.3, 59.3, 14.5. MS (CI method) 424 $(M + H)^+$, 406 (100%). HPLC (System 1) 99.89%. Anal. ($C_{19}H_{19}F_2N_3O_4S$) C: calc., 53.90; found, 54.12; H: calc., 4.52; found, 4.91; N: calc., 9.92; found, 9.59.

Compound 20. Preparation was from 4-chloroacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 72%. Mp 130–132 °C. IR (KBr) 3329, 1601 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.00 (d, $J = 8.6$ Hz, 1H, Ar), 7.59 (s, 1H, Ar), 7.36 (d, $J = 8.4$ Hz, 2H, Ar), 7.25 (s, 1H, Ar), 7.17 (d, $J = 7.0$ Hz, 2H, Ar), 6.75 (t, $J = 54.8$ Hz, 1H, CHF_2), 6.74 (s, 1H, C_4H), 5.44 (bs, 2H, SO_2NH_2), 5.01 (s, 2H, CH_2O), 2.80 (bs, 1H, OH). MS (CI method) 413 (M^+), 393 (100%), 378, 332. HPLC (System 1) 98.30%; (System 2) 98.50%. Anal. ($C_{17}H_{14}ClF_2N_3O_3S$) C: calc., 49.34; found, 49.55; H: calc., 3.41; found, 3.77; N: calc., 10.15; found, 10.39.

Compound 21. Preparation was from 4-bromoacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 68%. Mp 210–211 °C. IR (KBr) 3373, 1723, 1600, 1451 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.98 (d, $J = 8.6$ Hz, 1H, Ar), 7.59 (d, $J = 1.8$ Hz, 1H, Ar), 7.51 (d, $J = 8.2$ Hz, 2H, Ar), 7.20 (dd, $J = 6.4, 2.0$ Hz, 1H, Ar), 7.05 (d, $J = 9.4$ Hz, 2H, Ar), 6.76 (t, $J = 54.8$ Hz, 1H, CHF_2), 6.74 (s, 1H, C_4H), 5.49 (bs, 2H, SO_2NH_2), 5.02 (s, 2H, CH_2O), 4.25 (bs, 1H, OH). MS (CI method) 460 $(M + 2)^+$, 440, 378. HPLC (System 1) 98.60%; (System 2) 98.93%. Anal. ($C_{17}H_{14}BrF_2N_3O_3S$) C: calc., 44.56; found, 44.80; H: calc., 3.08; found, 3.32; N: calc., 9.17; found, 9.51.

Compound 22. Preparation was from 3-chloro-4-fluoroacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 69%. Mp 122–124 °C. IR (KBr) 3349, 1602, 1462, 1424 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.03 (d, $J = 8.5$ Hz, 1H, Ar), 7.61 (s, 1H, Ar), 7.42–7.05 (m, 4H, Ar), 6.77 (s, 1H, C_4H), 6.77 (t, $J = 53.2$ Hz, 1H, CHF_2), 5.05 (d, $J = 3.4$ Hz, 2H, CH_2O). MS (CI method) 431 (M^+), 412, 396, 350. HPLC (System 1) 99.23%. Anal. ($C_{17}H_{13}ClF_3N_3O_3S$) C: calc., 47.29; found, 46.95; H: calc., 3.03; found, 3.28; N: calc., 9.73; found, 9.52.

Compound 23. Preparation was from 4-methylaminoacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 71%. Mp 149–151 °C. IR 3431, 3323, 1617 cm^{-1}. ^1H NMR ($CD_3OD + CDCl_3$): δ 7.95 (d, $J = 8.4$ Hz, 1H, Ar), 7.73 (d, $J = 2.4$ Hz, 1H, Ar), 7.24 (dd, $J = 8.4, 2.4$ Hz, 1H, Ar), 7.01 (d, $J = 8.4$ Hz, 2H, Ar), 6.64 (t, $J = 54.8$ Hz, 1H, CHF_2), 6.56 (s, 1H, C_4H), 6.52 (d, $J = 8.8$ Hz, 2H, Ar), 5.00 (s, 2H, CH_2O), 2.82 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 150.4, 146.6 (t, $J = 28.8$ Hz, 1C, C-4), 145.8, 142.5, 142.3, 139.2, 129.6 (2C), 127.9, 123.6, 122.9, 115.4, 111.5 (t, $J = 231.4$ Hz, 1C, CHF_2), 111.4 (2C), 104.2, 59.4, 29.4. MS (CI method) 408 (M^+), 389 (100%), 356. HPLC (System 1) 98.20%; (System 2) 97.60%. Anal. ($C_{18}H_{18}F_2N_4O_3S$) C: calc., 52.94; found, 53.59; H: calc., 4.44; found, 4.75; N: calc., 13.72; found, 12.95.

Compound 24. Preparation was from 4-dimethylaminoacetophenone and ethyldifluoro acetate following steps 2 and 3. Yield 76%. Mp 89–91 °C. IR (KBr) 3460, 3320, 3245, 1618 cm^{-1}. ^1H NMR (CD_3OD): δ 7.98 (d, $J = 6.8$ Hz, 1H, Ar), 7.80 (s, 1H, Ar), 7.25 (dd, $J = 1.6, 4.8$ Hz, 1H, Ar), 7.04 (d, $J = 7.8$ Hz, 2H, Ar), 6.77 (t, $J = 54.8$ Hz, 1H, CHF_2), 6.75 (d, $J = 6.6$ Hz, 2H, Ar), 6.62 (s, 1H, C_4H), 5.02 (s, 2H, CH_2O), 2.95 (s, 6H, 2 × CH_3). MS (CI method) 422 (M^+), 405, 340, 312. HPLC (System 1) 98.89%. Anal.

($C_{19}H_{20}F_2N_4O_3S$) C: calc., 54.02; found, 53.87; H: calc., 4.77; found, 4.58; N: calc., 13.26; found, 13.52.

Compound 25. Preparation was from 3,4-dimethylacetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 70%. Mp 140–142 °C. IR (KBr) 3403, 1603 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.93 (d, J = 8.6 Hz, 1H, Ar), 7.60 (s, 1H, Ar), 7.26–7.10 (m, 2H, Ar), 7.05 (s, 1H, Ar), 6.85 (d, J = 7.4 Hz, 1H, Ar), 6.77 (s, 1H, C_4H), 6.69 (t, J = 54.8 Hz, 1H, CHF_2), 5.55 (s, 2H, SO_2NH_2), 5.00 (s, 2H, CH_2O), 2.28 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.92 (bs, 1H, OH). MS (CI method) 407 (M$^+$), 389, 361. HPLC (System 1) 97.41%. Anal. ($C_{19}H_{19}F_2N_3O_3S$) C: calc., 56.01; found, 56.12; H: calc., 4.70; found, 4.89; N: calc., 10.31; found, 10.56.

Compound 26. Preparation was from 3-methyl-4-methoxy-acetophenone and ethyl difluoroacetate following steps 2 and 3. Yield 65%. Mp 164–166 °C. IR (Neat) 3452, 3271, 1635, 1605, 1462 cm^{-1}. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 7.95 (d, J = 8.4 Hz, 1H, Ar), 7.63 (s, 1H, Ar), 7.18 (d, J = 6.6 Hz, 1H, Ar), 7.07 (s, 1H, Ar), 6.98 (d, J = 8.8 Hz, 1H, Ar), 6.94 (d, J = 7.2 Hz, 1H, Ar), 6.77 (t, J = 54.8 Hz, 1H, CHF_2), 6.64 (s, 1H, C_4H), 6.45 (s, 2H, SO_2NH_2), 4.95 (s, 2H, CH_2O), 3.84 (s, 3H, OCH_3), 2.38 (bs, 1H, OH), 2.18 (s, 3H, CH_3). MS (CI method) 423 (M$^+$), 403. HPLC (System 2) 97.89%. Anal. ($C_{19}H_{19}F_2N_3O_4S$) C: calc., 53.90; found, 53.81; H: calc., 4.52; found, 4.83; N: calc., 9.92; found, 10.21.

Compound 27. Preparation was from 2,3-dihydrobenzo[*b*]furan and ethyl difluoroacetate following steps 1–3. Yield 59%. Mp 76–78 °C. IR (KBr) 3374, 1602 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.93 (d, J = 8.4 Hz, 1H, Ar), 7.59 (d, J = 1.6 Hz, 1H, Ar), 7.20 (d, J = 6.6 Hz, 1H, Ar), 7.05 (s, 1H, Ar), 6.92 (d, J = 8.0 Hz, 1H, Ar), 6.71 (d, J = 6.6 Hz, 1H, Ar), 6.66 (t, J = 55.4 Hz, 1H, CHF_2), 6.47 (s, 1H, C_4H), 5.53 (bs, 2H, SO_2NH_2), 4.99 (s, 2H, CH_2O), 4.61 (t, J = 8.8 Hz, 2H, OCH_2CH_2), 3.18 (t, J = 8.8 Hz, 2H, OCH_2CH_2), 3.05 (bs, 1H, OH). MS (CI method) 421 (M$^+$), 403 (100%), 322. HPLC (System 2) 99.23%. Anal. ($C_{19}H_{17}F_2N_3O_4S$) C: calc., 54.15; found, 54.22; H: calc., 4.07; found, 3.82; N: calc., 9.97; found, 10.21.

Compound 28. Preparation was from indan and ethyl difluoro-acetate following steps 1–3. Yield 48%. Mp 99–100 °C. IR (KBr) 3384, 1602 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.92 (d, J = 7.6 Hz, 1H, Ar), 7.63 (s, 1H, Ar), 7.30–7.05 (m, 3H, Ar), 6.92 (d, J = 7.4 Hz, 1H, Ar), 6.67 (t, J = 54.8 Hz, 1H, CHF_2), 6.50 (s, 1H, C_4H), 5.50 (bs, 2H, SO_2NH_2), 5.00 (s, 2H, CH_2O), 3.00–2.82 (m, 4H, 2 × CH_2), 2.50 (bs, 1H, OH), 2.20–2.00 (m, 2H, CH_2). MS (CI method) 419 (M$^+$), 401 (100%), 320. HPLC (System 2) 97.15%. Anal. ($C_{20}H_{19}F_2N_3O_3S$) C: calc., 57.27; found, 57.31; H: calc., 4.57; found, 4.80; N: calc., 10.02; found, 10.23.

Computational methods

All molecular modeling studies were performed using the SYBYL program package, version 6.9[30] on Silicon Graphics Octane 2 work-station with the IRIX 6.5 operating system. The crystal structure of murine COX-2 (6COX, 87% homology with human) with SC-558 was used in this study.[15] Owing to the position of the NSAID binding site, only one monomer was considered in the calculation. Docked molecules were pre-aligned with respect to SC-558 in the active site of COX-2 using FIT-ATOMS in SYBYL. A manual docking procedure was adopted where hydrogen atoms were added to the proteins while all the residues were considered in the neutral form. The haeme, bound at the peroxidase active site, was removed since the majority of the molecules exceeded the cutoff radius. The active site was minimized (5.5 Å around the ligand) using the MMFF94[31] force field. The ligand–enzyme assembly was then subjected to a molecular dynamics (MD) treatment at a constant temperature of 300 K for 100 ps with a time step of 1 fs using a distance-dependent dielectric constant 4*r*. The resultant minimum energy structures obtained after molecular dynamics studies were re-minimized using the MMFF94 force field and were analyzed for ligand–receptor interactions in the active site.

In-vitro enzyme assay (ref. 23)

The microsomal fraction of ram seminal vesicles was used as a source of the COX-1 enzyme, and microsomes from Sf-9 cells infected with baculovirus expressing human COX-2 cDNA were used as a source of the COX-2 enzyme in measuring the inhibitory activity by the TMPD method. The assay mixture (1000 µL) contained 100 µM Tris pH 8.0, 3 µM EDTA, 15 µM hematin, 150 units enzyme and 8% DMSO. The mixture was incubated at 25 °C for 15 min before initiation of enzyme reaction in the presence of the compound/vehicle. The reaction was initiated by the addition of 100 µM arachidonic acid and 120 µM TMPD, and the velocity of TMPD oxidation over the first 25 s was monitored at 603 nm. The IC_{50} values were calculated using non-linear regression analysis of percentage inhibitions.

In-vivo screening

Carrageenan-induced rat paw edema (ref. 24). Male wistar rats (120–140 g) were fasted for 16 h before starting the experiment. Compounds were suspended in 0.25% CMC and administered orally in a volume of 10 mL kg^{-1}. After 2 h of dosing, 50 µL of 1% λ-carrageenan, suspended in saline, was injected into the plantar aponeurosis of the right paw. The paw volume was measured 3 h before and after the carrageenan injection using a plethysmometer (Ugo-Basile, Italy). The paw edema was compared with the vehicle control group, and the percentage inhibition was calculated. ED_{50}s were calculated using a linear regression plot.

Endotoxin-induced pyresis in rats (ref. 25). Male wistar rats (150–170 g) were fasted for 16 h before starting the experiment, and the baseline rectal temperature was recorded with a flexible temperature probe (YSI series-400) connected to a digital thermometer. At time zero, the rats were injected with 0.36 mg kg^{-1} of lipopolysaccharide (LPS, Sigma Chemical Co., St. Louis, USA) intra-peritoneally, and the rectal temperatures were recorded after 5 and 7 h. The test compounds were administered 5 h after LPS injection to determine their antipyretic potential. The percentage reversal of pyrexia was calculated by taking the ratio of the difference in temperature at 5th and 7th hour and the baseline of the treated and the control groups.

Carrageenan-induced rat paw hyperalgesia (Randal–Selitto method, ref. 26). Hyperalgesia was induced in the hind paw of male wistar rats (150–170 g) by intraplantar injection of carrageenan (2 mg per paw). Test compounds were dosed after 2 h from the carrageenan injection. The vocalization response to compression of the carrageenan-injected paw was measured 1 h later by analgesiometer (Ugo-Basile, Italy). For normal response, one group of animals was given an intraplantar injection of saline. The percent increase in pain was calculated as difference in threshold in the treated group *versus* the control group. ED_{50}s were calculated using a linear regression plot.

Rat adjuvant-induced arthritis (ref. 27). Arthritis was induced by subplantar injection of 0.5 mg of *Mycobacterium butyricum* in light mineral oil into the right hind footpad of male wistar rats (140–160 g). Treatment was carried out in either a therapeutic or prophylactic manner. In the therapeutic treatment, dosing commenced after 14 days from adjuvant injection, *i.e.* after establishment of the disease in culled groups, and continued until the 30th day. Foot volumes of both injected and contralateral paws were determined on various days. Radiographs were taken on days 14 and 30 for all the groups. In the prophylactic treatment, dosing started on day 0 (day of adjuvant injection) and continued for 21 days. Foot volume, body weight and food consumption were determined on various days. Radiographs were taken on the last day of treatment. To assess the tibiotarsal joint integrity, radiographic scores were assigned based on soft tissue swelling, bone erosion, periosteal reaction and joint space destruction on an arbitrary scale of 0–3 by a radiologist who was blind to the treatment. The ED_{50}s were calculated at the end

of the treatment compared with the control group using a linear regression plot, based on foot volume of the injected paw.

Single dose pharmacokinetic studies. All of the studies were carried out in male wistar rats obtained from the National Institute of Nutrition, Hyderabad, India. The animals (200–225 g) were fasted for 12 h before starting the experiment and had free access to water throughout. The animals were fed 3 h after drug administration. The animals were dosed at 10 mg kg^{-1} (po) as a 0.25% CMC suspension, and 0.4 mL blood samples were collected into heparinized microfuge tubes at pre-determined time points from the retro-orbital plexus. The samples were analyzed by HPLC after a suitable extraction procedure, and plasma concentration *versus* time profiles were generated for the interesting compounds along with celecoxib. Pharmacokinetic parameters were calculated by non-compartmental model analysis.

Acknowledgements

The authors are thankful to Dr K. Anji Reddy and Professorr J. Iqbal for their constant support and encouragement, and to Analytical Research group, DRL, for spectral analysis.

References

1 D. Picot, P. J. Loll and R. M. Garavito, *Nature*, 1994, **367**, 243–249; T. Hla and K. Neilson, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 7384–7388; J. R. Vane, Y. S. Bakhle and R. M. Botting, *Annu. Rev. Pharmacol. Toxicol.*, 1998, **38**, 97–120; J. L. Wallace, *Am. J. Med.*, 1999, **107**, 11S–17S.
2 S. Moncada, R. Gryglewski, S. Bunting and J. R. Vane, *Nature*, 1976, **263**, 663–665; B. J. R. Whittle, G. A. Higgs, K. E. Eakins, S. Moncada and J. R. Vane, *Nature*, 1980, **284**, 271–273.
3 M. C. Allison, A. G. Howatson, C. J. Torrance, F. D. Lee and R. I. G. Russel, *New England J. Med.*, 1992, **327**, 749–754.
4 J. Vane, *Nature*, 1994, **367**, 215–216; W. Xie, D. L. Robertson and D. L. Simmons, *Drug Dev. Res.*, 1992, **25**, 249–265.
5 J. Meyer-irchrath and K. Schror, *Curr. Med. Chem.*, 2000, **7**, 1121–1129 and refs. cited therein; D. E. Griswold and J. L. Adams, *Med. Res. Rev.*, 1996, **16**, 181–206; D. L. DeWitt, *Mol. Pharmacol.*, 1999, **55**, 625–631.
6 T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. M. Veenhuizen, Y. Y. Zhang and P. C. Isakson, *J. Med. Chem.*, 1997, **40**, 1347–1365 and refs. cited therein.
7 P. Prasit, Z. Wang, C. Brideau, C. C. Chan, S. Charleson, W. Cromlish, D. Either, J. F. Evans, A. W. Ford-Hutchinson, J. Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G. P. O. Neil, M. Ouellet, M. D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Therien, P. Vickers, E. Wong, L. J. Xu, R. N. Young, R. Zamboni, S. Boyce, N. Rupniak, M. Forrest, D. Visco and D. Patrick, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1773–1778.
8 J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, R. S. Rogers, A. F. Shaffer, Y. Y. Zhang, B. S. Zweifel and K. Seibert, *J. Med. Chem.*, 2000, **43**, 775–777.
9 D. Riendeau, M. D. Percival, C. Brideau, S. Charleson, D. Dube, D. Ethier, J. P. Falgueyret, R. W. Friesen, R. G. GordonGreig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I. W. Rodger, M. Gresser, A. W. Ford-Hutchinson, R. N. Young and C. C. Chan, *J. Pharmacol. Exp. Ther.*, 2001, **296**, 558–566; L. A. Sorbera, R. M. Castaner, J. Silvestre and J. Castaner, *Drugs Fut.*, 2001, **26**, 346–353.
10 K. E. Giercksky, *Best Pract. Res. Clin. Gastroenterol.*, 2001, **15**, 821–833; H. Vainio, *Int. J. Cancer*, 2001, **94**, 613–614; A. S. Kalgutkar and Z. Zhao, *Curr. Drug Targets*, 2001, **2**, 79–106.
11 G. M. Pasinetti, *J. Neurosci. Res.*, 1998, **54**, 1–6.
12 N. V. Chandrasekharan, H. Dai, K. L. Roos, N. K. Evanson, J. Tomsik, T. S. Elton and D. L. Simmons, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 13 371.
13 D. Mukherjee, S. E. Nissen and E. J. Topol, *J. Am. Med. Assoc.*, 2001, **286**, 954–959.
14 R. Xavier, *Drugs Today*, 1996, **32**, 365–384; G. Cignarella, P. Vianello, F. Berti and G. Rossoni, *Eur. J. Med. Chem.*, 1996, **31**, 359–364.
15 R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, R. A. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson and W. C. Stallings, *Nature*, 1996, **384**, 644–648.
16 (*a*) D. J. P. Pinto, D. G. Batt, W. J. Pitts, J. J. Petraitis, M. J. Orwat, S. Wang, J. W. Jetter, S. R. Sherk, G. C. Houghton, R. A. Copeland, M. B. Covington, J. M. Trzaskos and R. L. Magolda, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 919–924; (*b*) J. J. Li, M. B. Norton, E. J. Reinhard, G. D. Anderson, S. A. Gregory, P. C. Isakson, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, K. Seibert, Y. Zhang, B. S. Zweifel and D. B. Reitz, *J. Med. Chem.*, 1996, **39**, 1846–1856; (*c*) R. P. N. Praveen, M. Amini, H. Li, G. Habeeb and E. E. Knaus, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2205–2209; (*d*) Y. H. Joo, J. K. Kim, S. H. Kang, M. S. Noh, J. Y. Ha, J. K. Chio, K. M. Lim, C. H. Lee and S. Chung, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 413–417; (*e*) C. S. Li, C. Brideau, C. C. Chan, C. Savoie, D. Claveau, S. Charleson, R. Gordon, G. Greig, J. Y. Gauthier, C. K. Lau, D. Riendeau, M. Therien, E. Wong and P. Prasit, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 597–600; (*f*) J. S. Carter, S. Kramer, J. J. Talley, T. Penning, P. Collins, M. J. Graneto, K. Seibert, C. M. Koboldt, J. Masferrer and B. Zweifel, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1171–1174.
17 B. Baruah, D. Kavitha, V. Balasubramanian, V. Akhila, C. S. Rao and Y. K. Rao, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 445–448 and refs. cited therein.
18 J. Li, K. M. L. DeMello, H. Cheng, S. M. Sakya, B. S. Bronk, R. J. Rafka, B. H. Jaynes, C. B. Ziegler, C. Kilroy, D. W. Mann, E. L. Nimz, M. P. Lynch, M. L. Haven, N. L. Kolosko, M. L. Minich, C. Li, J. K. Dutra, B. Rast, R. M. Crosson, B. J. Morton, G. W. Kirk, K. M. Callaghan, D. A. Koss, A. Shavnya, L. A. Lund, S. B. Seibel, C. F. Petras and A. Silvia, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 95–98.
19 H. Hashimoto, K. Imamura, J. I. Haruta and K. Wakitani, *J. Med. Chem.*, 2002, **45**, 1511–1517; M. Pal, M. Madan, P. Srinivas, V. R. Pattabiraman, K. Srinivas, V. Akhila, M. Ramesh, N. V. S. R. Mamidi, R. C. Seshagiri, M. Alpeshkumar, B. Gopalakrishnan and Y. K. Rao, *J. Med. Chem.*, 2003, **46**, 3975–3984.
20 S. K. Singh, P. G. Reddy, K. S. Rao, B. B. Lohray, P. Misra, S. A. Rajjak, Y. K. Rao and A. Venkateswarlu, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 499–504.
21 W. Adcock, B. D. Gupta and T. C. Khor, *J. Org. Chem.*, 1976, **41**, 751–759.
22 Unpublished results.
23 (*a*) W. A. Cromlish, P. Payette, S. A. Culp, M. Ouellet, M. D. Percival and B. P. Kennedy, *Arch. Biochem. Biophys.*, 1994, **314**, 193–199; (*b*) C. C. Chan, S. Boyce, C. Brideau, S. Charleson, W. Cromlish, D. Ethier, J. Evans, A. W. Ford-Hutchinson, M. J. Forrest, J. Y. Gauthier, R. Gordon, M. Gresser, J. Guay, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G. P. O'Neill, M. Ouellet, D. Patrick, M. D. Percival, H. Perrier, P. Prasit, I. Rodger, P. Tagari, M. Therien, P. Vickers, D. Visco, Z. Wang, J. Webb, E. Wong, L. J. Xu, R. N. Young, R. Zamboni and D. Riendeau, *J. Pharmacol. Exp. Ther.*, 1999, **290**, 551–560.
24 C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 1962, **111**, 544–547.
25 D. S. Kosersky, W. L. Dewey and L. S. Harris, *Eur. J. Pharmacol.*, 1973, **24**, 1–7; S. W. Hajare, S. Chandra, S. K. Tandan, J. Sarma, J. Lal and A. G. Telang, *Indian J. Pharmacol.*, 2000, **32**, 357–360.
26 K. Hargreaves, R. Dubner, F. Brown, C. Flores and J. A. Joris, *Pain*, 1988, **32**, 77–88; K. Seibert, Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee and P. Isakson, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 12 013–12 017.
27 B. D. Jaffee, J. S. Kerr, E. A. Jones, J. V. Giannaras, M. McGowan and N. R. Ackerman, *Agents Actions*, 1989, **27**, 344–346.
28 S. L. Marks and D. A. Williams, *Am. J. Vet. Res.*, 1998, **59**, 1113–1115.
29 J. M. Young, S. Panah, C. Satchawatcharaphong and P. S. Cheung, *Inflammation Res.*, 1996, **45**, 246–253; C. Brideau, S. Kargman, S. Liu, A. L. Dallob, E. W. Ehrich, I. W. Rodger and C. C. Chan, *Inflammation Res.*, 1996, **45**, 68–74.
30 *SYBYL 6.9 Molecular Modeling Software*, Tripos Associates Inc., 1699 South Hanley Road, St. Louis, MO 63144, USA.
31 T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490–519.